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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
REDDIG, PETER J	
ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 10/17/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/537,002	SAHIN ET AL.	
	Examiner	Art Unit	
	Peter J. Reddig	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 August 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *99-115* is/are pending in the application.

4a) Of the above claim(s) *107-115* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *99-106* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *8/4/05, 5/20/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *Appendix 1 and 2*.

DETAILED ACTION

1. The Election filed on 8/02/06 in response to the Office Action of 06/02/06 is acknowledged and has been entered.

Applicant's election with traverse of Group III, claims 99-106, drawn to a technical feature of a method of diagnosing a disease, comprising detecting a polypeptide tumor associated antigen, wherein said antigen has a sequence encoded by SEQ ID NO: 7 is acknowledged and entered.

Applicant traverses the *a posteriori* finding of lack of a unifying special technical feature based on the reference WO 99/664452. Applicant argues that the reference relates to GPR35A not claudin-18A1 and claudin-18A2 of the instant claims. Applicant argues that claims 99-115 are linked by a new and inventive common special technical feature. Applicant argues that the inventive step of the instant invention relates to an appreciation that the expression of certain molecules is increase in a diseased state. Applicant argues that detection of the nucleic acid encoding the same tumor-associated antigen could also be searched without a serious burden to the Examiner. Applicant argues that both the nucleic acids (SEQ ID NO: 7 and 117) and polypeptides (SEQ ID NO: 16 and 118) of the instant invention are elevated in the disease state. Applicant further argues that SEQ ID NO: 16 and 118 could be searched together without serious burden to the Examiner because they are splice variants of the claudin-18A polypeptide, are encoded by the same gene, and differ only in the N-terminal extracellular domain.

The argument that WO 99/664452 relates to GPR35A not claudin-18A1 or claudin-18A2 of the instant invention has been considered and found persuasive.

Applicant argues that the special technical feature linking Groups I-XII is the inventive step concerning an appreciation that detection of certain molecules are increased in a diseased state. However, a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept. Unity of invention is fulfilled only when there is a technical relationship among the inventions involving one or more of the same or corresponding special technical features which define a contribution over the prior art. If there is no special technical feature, if multiple products, processes of manufacture or uses are claimed, the first invention of the category first mentioned in the claims of the application will be considered as the main invention in the claims, see PCT article 17(3) (a) and 1.476 (c), 37 C.F.R. 1.475(d). After that, all other products and methods will be broken out as separate groups (see 37 CFR 1.475(d).)

The inventions listed as Groups I-XII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Applicant argues that the special technical feature linking Groups I-XII is the inventive step concerning an appreciation that detection of certain molecules are increased in a diseased state.

However, Rosen et al. (WO 01/54708 A1) teaches a method of diagnosing a disease characterized by aberrant expression of a tumor associated Claudin-18A2 wherein Claudin-18A2 is at a higher amount in the diseased tissue, see p. 8-11, Table 1 and SEQ ID NO: 68, and appendix 1.

Therefore, the issue remains the same, the technical feature linking the inventions of Groups I-XII does not constitute a special technical feature as defined by PCT Rule 13.2 as it does not define a contribution over the prior art, thus each of the processes of use are properly broken out as separate groups.

Although applicant argues that detection of the nucleic acids encoding the same tumor associated antigens could also be searched without a serious burden of search, burden of search is not the criteria for proper restriction under PCT article 17(3) (a) and 1.476 (c), 37 C.F.R. 1.475(d). Given that the inventions lack unity, for the reasons set forth above, the restriction requirement is deemed to be proper and is therefore made FINAL.

2. Upon review and reconsideration and it is found that the instantly claimed Group III contains claims drawn to the following additional distinct Groups:

Group III-1, claim(s) 99-104 and 106, drawn to a technical feature of a method of diagnosing a disease, comprising detecting a polypeptide tumor associated antigen, wherein said antigen has a sequence encoded by SEQ ID NO: 7.

Group III-2, claim(s) 99 and 105, drawn to the method as claimed in claim 99, in which the disease is characterized by expression or abnormal expression of two or more different tumor-associated antigens and in which detection comprises detection of two or more different tumor associated antigens or portions thereof, drawn to detecting polypeptides.

Applicant is required to elect a specific combination of two or more than two different tumor associated antigens for examination.

Group III-3 claim(s) 99 and 105, drawn to the method as claimed in claim 99, in which the disease is characterized by expression or abnormal expression of two or more different tumor-associated antigens and in which detection comprises detection of two or more different tumor associated antigens or portions thereof, drawn to detecting nucleic acids.

Applicant is required to elect a specific combination of two or more than two different tumor associated antigens for examination.

During a telephone conversation with Melissa Wenk on September 25, 2006 a provisional election was made without traverse to prosecute the invention of Group III-1 claim(s) 99-104,and 106 drawn to a technical feature of a method of diagnosing a disease, comprising detecting a polypeptide tumor associated antigen, wherein said antigen has a sequence encoded by the nucleic acid of SEQ ID NO: 7. Examiner agreed to examine these claims as also drawn to a polypeptide encoded by the nucleic acid SEQ ID NO: 117 because of the substantial similarity of these splice variants. Affirmation of this election must be made by applicant in replying to this Office action.

3. Upon review and reconsideration Examiner will also examiner Group III-1 as drawn to a polypeptide of SEQ ID NO: 16 or 118 as the polypeptides are respectively encoded by SEQ ID NO: 7 and 117. Examiner will also examine Group III-1 as drawn to a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ ID NO: 7 or 117 or a portion thereof. Further, claim 105, only as it is drawn to assay of two different tumor associated antigens, the antigens encoded by SEQ ID NO: 7 and SEQ ID NO: 117 is hereby rejoined to the inventive group.

4. Claims 99-115 are pending.

5. Claim 105 as it is drawn to two antigens other than those encoded by SEQ ID NO: 7, SEQ ID NO: 117 and as it is drawn to more than two antigens, and claims 107-115 have been withdrawn from further consideration by the examiner under 37 CFR 1.142(b) as being drawn to non-elected inventions.

6. Claims 99-106 as drawn to the technical features of a method of diagnosing a disease by detecting expression or abnormal expression of a polypeptide encoded by a

nucleic acid of SEQ ID NO: 7 or 117 a polypeptide of SEQ ID NO: 16 or 118 are currently under consideration.

Specification

7. The abstract is objected to because of the following informalities: The use of the word said, see MPEP 608.01(b).

Appropriate correction is required.

Applicant is reminded of the proper language and format for an abstract of the disclosure.

The abstract should be in narrative form and generally limited to a single paragraph on a separate sheet within the range of 50 to 150 words. It is important that the abstract not exceed 150 words in length since the space provided for the abstract on the computer tape used by the printer is limited. The form and legal phraseology often used in patent claims, such as "means" and "said," should be avoided. The abstract should describe the disclosure sufficiently to assist readers in deciding whether there is a need for consulting the full patent text for details.

The language should be clear and concise and should not repeat information given in the title. It should avoid using phrases which can be implied, such as, "The disclosure concerns," "The disclosure defined by this invention," "The disclosure describes," etc.

8. The disclosure is objected to because of the following informalities: The arrangement of the specification is incorrect, see MPEP 601. The titles for the Title of the Invention, Background of the Invention, and Brief Summary of the Invention sections are missing. The Figures should be labeled as Brief Description of the Several Views of the Drawings and this section should follow the Brief Summary of the Invention section.

Appropriate correction is required.

The following guidelines illustrate the preferred layout for the specification of a utility application. These guidelines are suggested for the applicant's use.

Arrangement of the Specification

As provided in 37 CFR 1.77(b), the specification of a utility application should include the following sections in order. Each of the lettered items should appear in upper case, without underlining or bold type, as a section heading. If no text follows the section heading, the phrase "Not Applicable" should follow the section heading:

(a) TITLE OF THE INVENTION.
(b) CROSS-REFERENCE TO RELATED APPLICATIONS.
(c) STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT.
(d) THE NAMES OF THE PARTIES TO A JOINT RESEARCH AGREEMENT.
(e) INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ON A COMPACT DISC.
(f) BACKGROUND OF THE INVENTION.
    (1) Field of the Invention.
    (2) Description of Related Art including information disclosed under 37 CFR 1.97 and 1.98.
(g) BRIEF SUMMARY OF THE INVENTION.
(h) BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S).
(i) DETAILED DESCRIPTION OF THE INVENTION.
(j) CLAIM OR CLAIMS (commencing on a separate sheet).
(k) ABSTRACT OF THE DISCLOSURE (commencing on a separate sheet).
(l) SEQUENCE LISTING (See MPEP § 2424 and 37 CFR 1.821-1.825. A "Sequence Listing" is required on paper if the application discloses a nucleotide or amino acid sequence as defined in 37 CFR 1.821(a) and if the required "Sequence Listing" is not submitted as an electronic document on compact disc).

Priority

9. It is noted that examiner has established a priority date for the instant application, 10/537,002, of May 20, 2005 because the priority of the instantly claimed invention is based on the PCT and German patent applications, numbers PCT/EP03/13091 and 102 54 601.0 (respectively), which have not been translated and examiner is unable to determine the information in the document. If applicant disagrees with any rejection set forth in this

action based on examiner's establishment of a priority date, May 20, 2005, for the instantly claimed application serial number 10/537,002, applicant is invited to submit a proper translation of the priority document and to point to, page and line where support can be found establishing an earlier priority date.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

10. Claim 103 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 103 recites the limitation "the detectable marker" in the method as claimed in claim 102. There is insufficient antecedent basis for this limitation in the claim.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

11. Claim 106 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claim is drawn to the method as claimed in claim 99, in which the tumor-associated antigen or portion thereof to be detected is in a complex with an MHC molecule.

This means that the tumor associated antigen, claudin-18A1 or claudin-18A2, or a portion thereof could be detected in a complex with an MHC molecule and this could be used for the diagnostic method of claim 99.

The specification teaches that tumor cell differences are due to genetic alterations acquired during tumor development and result in the formation of qualitatively or quantitatively altered molecular structures in the cancer cells. The specification teaches that tumor-associated structures of this kind which are recognized by the specific immune system of the tumor-harboring host are referred to as tumor-associated antigens. The specification teaches that the specific recognition of tumor-associated antigens involves cellular and humoral mechanisms which are two functionally interconnected units: $CD4^+$ and $CD8^+$ T lymphocytes recognize the processed antigens presented on the molecules of the MHC (major histocompatibility complex) classes II and I, respectively, see p. 1, lines 15-32. The specification teaches that, in further embodiments, the tumor-associated antigen to be detected or the part thereof is present in a complex with an MHC molecule, in particular an HLA molecule, p. 16, lines 14-16.

One cannot extrapolate the teachings of the specification to the enablement of the claims because one cannot predict based on the teachings of the specification which claudin-18A1 or claudin-18A2 peptides will be found in a complex with an MHC molecule and, thus, one cannot reasonably predict which peptides would be detectable

given the additional processing of the antigen before presentation with an MHC molecule.

In particular, Herbert et al (The Dictionary of Immunology, Academic Press, 3rd Edition, London, 1985, p. 58-59), teaches that T-cells recognize peptide fragments which have been processed by an accessory cell and presented in the cleft of a class I MHC antigen or a class II MHC antigen and that a continuous primary sequence is necessary for T cell recognition, see p. 58. As is appreciated in the art, the HLA Class I binding groove is essentially closed ended so that peptides of only a particular size range can fit into the groove and be bound, generally HLA Class I epitopes are 8, 9, 10, or 11 amino acids long. In contrast, the HLA Class II binding groove is essentially open ended; therefore a peptide of about 9 or more amino acids can be bound by an HLA Class II molecule.

Given the above, one could not reasonably predict which fragment of polypeptide of SEQ ID NO: 16 or 118 or a portion thereof, any polypeptide encoded by a nucleic acid of SEQ ID NO: 7 or 117 or a portion thereof, any polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ ID NO: 7 or 117 or a portion thereof, would be in complex with an MHC molecule. This is because these polypeptides have been processesed into small peptides and presented with an MHC molecule the without knowledge of which polypeptides would be presented with the MHC molecule. Thus, undue experimentation would be required to determine which polypeptides, the processing of the antigen before presentation with the MHC molecule, of the claimed method would be detectable in complex with an MHC molecule.

Applicant is reminded that MPEP 2164.03 teaches "the amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability of the art. In re Fisher, 428 F.2d 833, 166 USPQ 18, 24 (CCPA 1970) the amount of guidance or direction refers to that information in the application, as originally filed, that teaches exactly how to make or use the invention. The more that is known in the prior art about the nature of the invention, how to make, and how to use the invention, and the more predictable the art is, the less information needs to be explicitly state in the specification. In contrast, if little is known in the prior art about the nature of the invention and the art is unpredictable, the specification would need more detail as how to make and use the invention in order for it to be enabling. Given only lack of guidance in the specification, no one skilled in the art would accept the assertion that the claimed invention would function as contemplated or as claimed based only on the information in the specification and that known in the art at the time the invention was made.

The specification provides insufficient guidance with regard to these issues and provides no working examples which would provide guidance to one skilled in the art and no evidence has been provided which would allow one of skill in the art to predict that the invention will function as contemplated with a reasonable expectation of success. For the above reasons, it appears that undue experimentation would be required to practice the claimed invention.

12. If applicant were able to overcome the 35 U.S.C. 112, first paragraph rejection set forth above, Claims 99-106 would still be rejected under 35 U.S.C. 112, first paragraph,

as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claims are drawn to a method of diagnosing a disease characterized by overexpression of a tumor-associated antigen comprising detection of the tumor-associated antigen in a biological sample isolated from a patient, wherein the tumor-associated antigen is selected from the group consisting of: (i) a polypeptide of SEQ ID NO: 118; and (ii) a polypeptide encoded by a nucleic acid of SEQ ID NO: 117, (iii) a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ NO: 117, and in which the tumor-associated antigen to be detected is in a complex with an MHC molecule; wherein detection of the tumor-associated antigen in the biological sample in an amount greater than an amount of the tumor-associated antigen in a normal biological sample indicates the disease.

The specification teaches that the splice variants claudin-18A1 is SEQ ID NO: 117, and its translation product is SEQ ID NO: 118, p. 92, lines 21-24.

The specification teaches that, using quantitative PCR, claudin-18A1 mRNA expression levels in ENT, prostate, pancreatic and esophageal tumors are 100-10,000 times higher than the levels in the corresponding normal tissues (see Example 4, p. 93 and Fig. 23). For the claudin-18A1 protein, the specification teaches when using antibodies specific to claudin-18A1 that weak to absent staining of gastric carcinoma cells (SNU-16) in culture was observed, see Fig. 28.

One cannot extrapolate the teachings of the specification to the enablement of the claims because 1) there is no nexus established between claudin-18A1 protein overexpression in any disease state because the only data for abnormal expression of claudin-18A1 shown is at the mRNA level and the unpredictability of extrapolating between mRNA expression levels and protein expression levels is well known in the art 2) the expression of claudin-18A1 protein in gastric carcinoma cells (SNU-16) in cell culture is not predictive of *in vivo* gastric carcinoma expression because the artifactual nature of cell culture is well known in the art.

1) As drawn to claudin-18A1 expression, although the specification teaches that, using quantitative PCR, claudin-18A1 mRNA expression levels in ENT, prostate, pancreatic and esophageal tumors are 100-10 000 higher than the levels in the corresponding normal tissues (see Example 4, p. 93, Table 3B, and Fig. 23) it is well known in the art that RNA expression does not predictably correlate with protein expression.

In particular, as drawn to the unpredictability of determining protein expression based on RNA expression data, Greenbaum *et al.* (Genome Biology, 2003, Vol. 4, Issue 9, pages 117.1-117.8) cautions against assuming that mRNA levels are generally correlative of protein levels. The reference teaches (page 117.3, col 2) that primarily because of a limited ability to measure protein abundances, researchers have tried to find correlations between mRNA and the limited protein expression data, in the hope that they could determine protein abundance levels from the more copious and technically easier mRNA experiments. To date, however, there have been only a handful of efforts to find correlations between mRNA and protein expression levels, most notably in human

cancers and yeast cells. These studies, for the most part, have reported only minimal and/or limited correlations. The reference further teaches (page 117.4, col 2) that there are presumably at least three reasons for the poor correlations generally reported in the literature between the level of mRNA and the level of protein, and these may not be mutually exclusive. First, there are many complicated and varied post-transcriptional mechanisms involved in turning mRNA into protein that are not yet sufficiently well defined to be able to compute protein concentrations from mRNA; second, proteins may differ substantially in their *in vivo* half lives; and/or third, there is a significant amount of error and noise in both protein and mRNA experiments that limit our ability to get a clear picture. The reference further notes (page 117.6, col. 2) that to be fully able to understand the relationship between mRNA and protein abundances, the dynamic processes involved in protein synthesis and degradation have to be better understood.

Additional evidence abounds in which protein levels do not correlate with steady state mRNA levels or alterations in mRNA levels in both cancer and normal cell types. For instance, Brennan et al. (J. Autoimmunity, 1989, 2 (suppl.): 177-186) teach that high levels of the mRNA for TNF alpha were produced in synovial cells, but that the TNF alpha protein was undetectable. Zimmer (Cell Motility and the Cytoskeleton, 1991. 20:325-337) teaches that there is no correlation between the mRNA level of calcium-modulated protein S1000 alpha and the protein level, indicating that S100 protein is post-transcriptionally regulated. Hell et al. (Laboratory Investigation, 1995, 73: 492-496) teach that cell s in all types of Hodgkin's disease exhibited high levels of Bcl-2 mRNA, while the expression of the Bcl-2 protein was not homogenous to said cells. In addition, Fu et al. (EMBO J., 1996, 15:43982-4401) teach that levels of p53 protein expression do

not correlate with levels of p53 mRNA levels in blast cells taken from patients with acute myelogenous leukemia, said patients being without mutation in the p53 gene. Vallejo et al. (Biochimie, 2000 82:1129-1133) teach that no correlation was found between NRF-2 mRNA and protein levels suggesting post-transcriptional regulation of NRF-2 protein levels. These references serve to demonstrate that levels of RNAs cannot be relied upon to anticipate levels of protein. Further, Jang et al. (Clinical Exp. Metastasis, 1997, 15: 469-483) teach that further studies are necessary to determine if changes in protein levels track with changes in mRNA levels for a metastasis associated gene in murine tumor cells, thus providing further evidence that one of skill in the art cannot anticipate that the level of a specific mRNA expressed by a cell will be paralleled at the protein level due to complex homeostatic factors controlling translation and post-translational modification.

Thus, in the absence of objective evidence demonstrating that not only the claudin-18A1 mRNA, but also the claudin-18A1 protein, are expressed or abnormally expressed in primary tumor cells, one would not be able to predictably use the claimed invention for the contemplated method of diagnosing a disease based only on the measurement of claudin-18A1 mRNA levels to determine the expression of the claudin-18A1 protein as taught in the specification as originally filed.

2) As drawn to the detection of claudin-18A1 protein in gastric tumor cells in culture, the molecular artifacts associated with cell culture are well known in the art. For example, Drexler et al (Leukemia and Lymphoma, 1993, 9:1-25) specifically teach, in the study of Hodgkin and Reed-Sternberg cancer cells in culture, that the acquisition or loss of certain properties during adaptation to culture systems cannot be excluded. This is exemplified by the teachings of Zellner et al (Clin. Can. Res., 1998, 4:1797-17802)

who specifically teach that products are overexpressed in glioblastoma (GBM)-derived cell lines which are not overexpressed *in vivo*. Drexler et al further teach that only a few cell lines containing cells that resemble the *in-vivo* cancer cells have been established and even for the *bona fide* cancer cell lines it is difficult to prove that the immortalized cells originated from a specific cancer cell (see attached abstract). Further, Embleton et al (Immunol Ser, 1984, 23:181-207) specifically teaches that in procedures for the diagnosis of osteogenic sarcoma, caution must be used when interpreting results obtained with monoclonal antibodies that had been raised to cultured cell lines and specifically teach that cultured tumor cells may not be antigenically typical of the tumor cell population from which they were derived and it is well established that new artifactual antigens can occur as a result of culture (see attached abstract). Hsu (in Tissue Culture Methods and Applications, Kruse and Patterson, Eds, 1973, Academic Press, NY, see abstract, p.764) specifically teaches that it is well known that cell cultures *in vitro* frequently change their chromosomal constitutions (see abstract). Thus, based on the cell culture data presented in the specification, in the absence of data protein expression data for claudin-18A1 provided from primary tumor cells and normal controls, no one of skill in the art would believe it more likely than not that the claimed invention would function as claimed, that is a method of diagnosing disease based on the expression of claudin-18A1 protein, based only on the RNA expression and cell culture data provided for claudin-18A1.

Applicant is reminded that MPEP 2164.03 teaches "the amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability of the art. In re Fisher, 428 F.2d 833, 166 USPQ 18, 24 (CCPA 1970) the amount of guidance or direction refers to that

information in the application, as originally filed, that teaches exactly how to make or use the invention. The more that is known in the prior art about the nature of the invention, how to make, and how to use the invention, and the more predictable the art is; the less information needs to be explicitly state in the specification. In contrast, if little is known in the prior art about the nature of the invention and the art is unpredictable, the specification would need more detail as how to make and use the invention in order for it to be enabling. Given only lack of guidance in the specification, no one skilled in the art would accept the assertion that the claimed invention would function as contemplated or as claimed based only on the information in the specification and that known in the art at the time the invention was made.

The specification provides insufficient guidance with regard to these issues and provides no working examples which would provide guidance to one skilled in the art and no evidence has been provided which would allow one of skill in the art to predict that the invention will function as contemplated with a reasonable expectation of success. For the above reasons, it appears that undue experimentation would be required to practice the claimed invention.

13. If applicant were able to overcome the rejection set forth above, claims 99-106 would still be rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of diagnosing a **lung cancer** characterized by **overexpression** of a tumor-associated antigen comprising detection of the tumor-associated antigen in a biological sample isolated from a patient, wherein the tumor-associated antigen is selected from the group consisting of: (i) a polypeptide of SEQ ID

NO: 16; and (ii) a polypeptide encoded by a nucleic acid of SEQ ID NO:7; (iii) a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ NO: 7, and in which the tumor-associated antigen to be detected is in a complex with an MHC molecule ,wherein detection of the tumor-associated antigen in the biological sample in an amount greater than an amount of the tumor-associated antigen in a normal biological sample indicates **lung cancer**, does not reasonably provide enablement for a method of diagnosing **a disease** characterized by expression or abnormal expression of a tumor-associated antigen comprising detection of the tumor-associated antigen or a portion thereof in a biological sample isolated from a patient, wherein the tumor-associated antigen is selected from the group consisting of: (i) a polypeptide of SEQ ID NO: 16; and (ii) a polypeptide encoded by a nucleic acid of SEQ ID NO: 7; (iii) a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ NO: 7, and in which the tumor-associated antigen or portion thereof to be detected is in a complex with an MHC molecule ,wherein detection of the tumor-associated antigen in the biological sample in an amount greater than an amount of the tumor-associated antigen in a normal biological sample indicates the disease. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are drawn to a method of diagnosing a disease characterized by overexpresion of a tumor-associated antigen comprising detection of the tumor-associated antigen or a portion thereof in a biological sample isolated from a patient, wherein the tumor-associated antigen is selected from the group consisting of: (i) a polypeptide of SEQ ID NO: 16; and (ii) a polypeptide encoded by a nucleic acid of SEQ

ID NO: 7, (iii) a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ NO: 7, and in which the tumor-associated antigen or portion thereof to be detected is in a complex with an MHC molecule; wherein detection of the tumor-associated antigen in the biological sample in an amount greater than an amount of the tumor-associated antigen in a normal biological sample indicates the disease.

This means that the detection of the overexpression of a tumor associated antigen selected from a group consisting of a polypeptide of SEQ ID 16, a polypeptide encoded by a nucleic acid of SEQ ID NO: 7, or a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ NO: 7; and in which the tumor-associated antigen to be detected is in a complex with an MHC molecule, wherein any of the claimed tumor associated antigens is in a greater amount than found in a normal biological sample will allow one to diagnose disease.

The specification as originally filed appears to treat bronchial carcinomas and lung tumors as equivalent, thus for examination purposes when the specification teaches bronchial carcinomas or lung tumors it is assumed that they are equivalent, see Example 4,Table 3A and 3B, and Fig 23, 24, and 31. Clarification is required.

The specification teaches that the term "disease" refers to any pathological state in which tumor-associated antigens are expressed or abnormally expressed, p. 41, lines 26-28.

The specification teaches that according to the invention, the term "expression" is used in its most general meaning and comprises the production of RNA or of RNA and protein, p. 98, lines 10-12. The specification teaches that "abnormal expression" means according

to the invention that expression is altered, preferably increased, compared to the state in a healthy individual, p. 41, lines 29-31.

The specification teaches that claudin-18A2 is SEQ ID NO: 7 and its translation products is SEQ ID NO: 16, p. 92, lines 21-24.

The specification teaches that claudin-18A2 protein is detected only in stomach and in no other normal tissue, not even lung, see p. 98 lines 10-12 and Fig. 29. The specification teaches that the comparative antibody staining of stomach tumors and adjacent normal stomach tissue from patients surprisingly revealed that claudin-18 A2 has a smaller mass weight in all stomach tumors in which this protein is detected and a band also appears at this level when lysate of normal stomach tissue is treated with the deglycosylating agent PNGase F, see p. 98, lines 12-20 and Fig. 30. The specification teaches that whereas exclusively the glycosylated form of the A2 variant is detectable in all normal stomach tissues, A2 is detectable as such in more than 60% of the investigated gastric carcinomas, in particular exclusively in the deglycosylated form, p. 98, lines 20-25. However, the claudin-18A2 protein, in any glycosylation state, does not appear to be overexpressed in gastric carcinomas versus normal stomach tissue, see Fig. 30.

Further, the specification teaches that the A2 variant of claudin-18 protein is not detected in normal lung while bronchial carcinomas/lung tumors express only the deglycosylated variant of the claudin-18A2, see p. 98, lines 25-30 and Fig. 31.

One cannot extrapolate the teachings of the specification to the scope of the claims because no nexus has been established between overexpression of claudin-18A2 protein expression in any disease, except overexpression in bronchial carcinomas/lung tumors, and the heterogeneity of disease, in particular cancer, is well known in the art

In particular, as drawn to cancer heterogeneity, cancers comprise a broad group of malignant neoplasms divided into two categories, carcinoma and sarcoma. The carcinomas originate in epithelial tissues while sarcomas develop from connective tissues, see Taber's Cyclopedic Medical Dictionary (1985, F.A. Davis Company, Philadelphia, p. 274). Given that not all cancers originate from the same tissue types, it is expected and known that cancers originate from different tissue types have different structures as well as etiologies and would present differently. Thus, it would not be predictably expected that a nexus, for example drawn to a connection between claudin-18A2 and cancer diagnosis, would be established between two cancer types that arose from different tissue types. Further, it is well known that even two carcinomas that present on the same organ have significant differences in etiology and genetic constitution. For example, Busken, C et al, (Digestive Disease Week Abstracts and Itinerary Planner, 2003, abstract No:850), teach that there is a difference in COX-2 expression with respect to intensity, homogeneity, localization and prognostic significance between adenocarcinoma of the cardia and distal esophagus, suggesting that these two cancers have different etiology and genetic constitution (last five lines of the abstract). Furthermore Krontiris and Capizzi (Internal Medicine, 4th Edition, Editor-in-chief Jay Stein, Elsevier Science, 1994 Chapters 71-72, pages 699-715) teach that the various types of cancers have different causative agents, involve different cellular mechanisms, and, consequently, differ in treatment protocols. Given the above, it is clear that it is not possible to predictably extrapolate a correlation between claudin-18A2 and any other disease, other than bronchial carcinomas/lung tumors, based on the information in the specification and known in the art without undue experimentation.

Thus, one would not be able to predictably diagnose any disease characterized by expression of claudin-18A2 protein wherein claudin-18A2 protein is in greater amounts in a sample than in a normal sample, except for bronchial carcinomas/lung tumors, without undue experimentation to demonstrate that the expression or abnormal expression of claudin-18A2 is associated with any other disease except lung cancer.

Applicant is reminded that MPEP 2164.03 teaches "the amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability of the art. In re Fisher, 428 F.2d 833, 166 USPQ 18, 24 (CCPA 1970) the amount of guidance or direction refers to that information in the application, as originally filed, that teaches exactly how to make or use the invention. The more that is known in the prior art about the nature of the invention, how to make, and how to use the invention, and the more predictable the art is, the less information needs to be explicitly state in the specification. In contrast, if little is known in the prior art about the nature of the invention and the art is unpredictable, the specification would need more detail as how to make and use the invention in order for it to be enabling. Given only lack of guidance in the specification, no one skilled in the art would accept the assertion that the claimed invention would function as contemplated or as claimed based only on the information in the specification and that known in the art at the time the invention was made.

The specification provides insufficient guidance with regard to these issues and provides no working examples which would provide guidance to one skilled in the art and no evidence has been provided which would allow one of skill in the art to predict that the invention will function as contemplated with a reasonable expectation of

success. For the above reasons, it appears that undue experimentation would be required to practice the claimed invention.

14. Claims 99-106 are rejected under 35 USC 112, first paragraph, as lacking an adequate written description in the specification.

Claims 99-106 are drawn to a method of diagnosing a disease characterized by overexpression of a portion of a tumor-associated antigen comprising detection of said portion in a biological sample isolated from a patient, wherein the tumor-associated antigen is selected from the group consisting of: (i) a portion of SEQ ID NO:16 or 118; (ii) a polypeptide encoded by a portion of SEQ ID NO:7 OR 117 (iii) a portion of a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ ID NO:7 or 117, and a portion of said antigen that is detected in a complex with an MHC molecule. Although drawn to DNA arts, the findings in University of California v. Eli Lilly and Co., 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997) and Enzo Biochem, Inc. V. Gen-Probe Inc. are relevant to the instant claims. The Federal Circuit addressed the application of the written description requirement to DNA-related inventions in University of California v. Eli Lilly and Co., 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997). The court stated that "[a] written description of an invention involving a chemical genus, like a description of a chemical species, requires a precise definition, such as by structure, formula, [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." Id. At 1567, 43 USPQ2d at 1405. The court also stated that

> a generic statement such as "vertebrate insulin cDNA" or "mammalian insulin cDNA" without more, is not an adequate written description of the genus because

> it does not distinguish the genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus. A definition by function, as we have previously indicated, does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is.

Id. At 1568, 43 USPQ2d at 1406. The court concluded that "naming a type of material generally known to exist, in the absence of knowledge as to what that material consists of, is not a description of that material." Id.

Finally, the court addressed the manner by which a genus of cDNAs might be described. "A description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus." Id.

The Federal Circuit has recently clarified that a DNA molecule can be adequately described without disclosing its complete structure. See Enzo Biochem, Inc. V. Gen-Probe Inc., 296 F.3d 1316, 63 USPQ2d 1609 (Fed. Cir. 2002). The Enzo court adopted the standard that "the written description requirement can be met by 'show[ing] that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics ....i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. " Id. At 1324, 63 USPQ2d at 1613 (emphasis omitted, bracketed material in original).

The inventions at issue in Lilly and Enzo were DNA constructs per se, the holdings of those cases are also applicable to claims such as those at issue here. A

disclosure that does not adequately describe a product itself logically cannot adequately describe a method of using that product.

Thus, the instant specification may provide an adequate written description of (i) a portion of SEQ ID NO: 16 or 118; (ii) a polypeptide encoded by a portion of SEQ ID NO: 7 OR 117 (iii) a portion of a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ ID NO: 7 or 117, and a portion of said antigen that is detected in a complex with an MHC molecule useful for diagnosing a disease, per Lilly by structurally describing a representative number of portions of said polypeptides or by describing "structural features common to the members of the genus, which features constitute a substantial portion of the genus." Alternatively, per Enzo, the specification can show that the claimed invention is complete "by disclosure of sufficiently detailed, relevant identifying characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics."

In this case, the specification does not describe (i) a portion of SEQ ID NO: 16 or 118; (ii) a polypeptide encoded by a portion of SEQ ID NO: 7 OR 117 (iii) a portion of a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ ID NO: 7 or 117, and a portion of said antigen that is detected in a complex with an MHC molecule useful for diagnosing a disease required to practice the method of claim 99 in a manner that satisfies either the Lilly or Enzo standards. The specification does not provide the complete structure of a portion of said polypeptides nor any functional characteristics coupled with a known or disclosed correlation between structure and function. Although the specification discloses SEQ ID NO: 16 and 118, this does not provide a description

of (i) a portion of SEQ ID NO: 16 or 118; (ii) a polypeptide encoded by a portion of SEQ ID NO: 7 OR 117 (iii) a portion of a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ ID NO: 7 or 117, and a portion of said antigen that is detected in a complex with an MHC molecule useful for diagnosing a disease that would satisfy the standard set out in Enzo.

The specification also fails to describe (i) a portion of SEQ ID NO: 16 or 118; (ii) a polypeptide encoded by a portion of SEQ ID NO: 7 OR 117 (iii) a portion of a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ ID NO: 7 or 117, and a portion of said antigen that is detected in a complex with an MHC molecule useful for diagnosing a disease by the test set out in Lilly. The specification describes only a SEQ ID NO: 16 and 118. Therefore, it necessarily fails to describe a "representative number" of such species. In addition, the specification also does not describe "structural features common to the members of the genus, which features constitute a substantial portion of the genus."

Thus, the specification does not provide an adequate written description of (i) a portion of SEQ ID NO: 16 or 118; (ii) a polypeptide encoded by a portion of SEQ ID NO: 7 OR 117 (iii) a portion of a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ ID NO: 7 or 117, and a portion of said antigen that is detected in a complex with an MHC molecule useful for diagnosing a disease that is required to practice the claimed invention. Since the specification fails to adequately describe portions of said polypeptides, it also fails to adequately describe the method of diagnosing a disease involving their detection.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

15. Claims 99-104 are rejected under 35 U.S.C. 102(b) as being anticipated by Rosen et al. (WO 01/54708, 2 August 2001) (see also appendix 1).

The claims are drawn a method of diagnosing a disease characterized by expression or abnormal expression of a tumor-associated antigen comprising detection of the tumor-associated antigen or a portion thereof in a biological sample isolated from a patient, wherein the tumor-associated antigen is selected from the group consisting of: (i) a polypeptide of SEQ ID NO: 16 or 118 or a portion thereof; and (ii) a polypeptide encoded by a nucleic acid of SEQ ID NO:7 or 117 or a portion thereof; (iii) a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ NO: 7 or 117 or a portion thereof, wherein detection of the tumor-associated antigen in the biological sample in an amount greater than an amount of the tumor-associated antigen in a normal biological sample indicates the disease (Claim 99), the method as claimed in 99, in which the detection comprises (i) contacting the biological sample with an agent which binds specifically to the tumor-associated antigen or the portion there of ; and (ii) detecting a complex formed between the agent and the tumor associated antigen or the portion thereof (claim 100), the method of as claimed in claim 100, wherein the agent is an antibody (claim 101), the method as claimed in claim 100, where in the agent is labeled in a detectable manner (claim 102), the method as claimed in claim 102, wherein

the detectable marker is a radioactive marker or an enzymatic marker (103), the method as claimed in claim 99, wherein the biological sample comprise body fluid or body tissue (claim 104).

Rosen et al. teach using antibodies to a polypeptide encoded by SEQ ID NO: 7 (i.e. claudin-18A2), which is SEQ ID NO: 68 of WO 01/54708 (see appendix 1), for the diagnosis of disease, including lung, pancreatic, and testicular cancer (see p. 10, line 10 to p.11, line 5) wherein the polypeptide is at a higher level in diseased tissues than in normal tissues, see p. 9, lines 5-49 and p. 10, lines 15-32. Rosen et al. teach using a sample taken from health tissue or bodily fluid, see p. 10 lines 1-3. Rosen et al. teach using antibodies labeled with radioactive or enzymatic markers, see p. 145, lines 4-8.

16. Claims 99-104 are rejected under 35 U.S.C. 102(b) as being anticipated by Sheppard and Foley (WO 00/15659, 23 March 2000) (see also appendix 2).

The claims are drawn a method of diagnosing a disease characterized by expression or abnormal expression of a tumor-associated antigen comprising detection of the tumor-associated antigen or a portion thereof in a biological sample isolated from a patient, wherein the tumor-associated antigen is selected from the group consisting of: (i) a polypeptide of SEQ ID NO: 16 or 118 or a portion thereof; and (ii) a polypeptide encoded by a nucleic acid of SEQ ID NO:7 or 117 or a portion thereof; (iii) a polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid of SEQ NO: 7 or 117 or a portion thereof, wherein detection of the tumor-associated antigen in the biological sample in an amount greater than an amount of the tumor-associated antigen in a normal biological sample indicates the disease (Claim 99), the method as claimed in 99, in which the detection comprises (i) contacting the biological sample with an agent which

binds specifically to the tumor-associated antigen or the portion there of ; and (ii) detecting a complex formed between the agent and the tumor associated antigen or the portion thereof (claim 100), the method of as claimed in claim 100, wherein the agent is an antibody (claim101), the method as claimed in claim 100, where in the agent is labeled in a detectable manner (claim 102), the method as claimed in claim 102, wherein the detectable marker is a radioactive marker or an enzymatic marker (103), the method as claimed in claim 99, wherein the biological sample comprise body fluid or body tissue (claim 104).

Sheppard and Foley teach using antibodies to a polypeptide encoded by SEQ ID NO: 117 (i.e. claudin-18A1), which is SEQ ID NO: 2 (zSig 28, see p. 15, lines 23-28) of WO 00/15659 (see appendix 2), for the diagnosis of disease, including stomach cancer (see p. 72 line 14-19) wherein the polypeptide is at a higher level in diseased tissues than in normal tissues, see p. 75, lines 1-26. Sheppard and Foley teach using a sample taken from tissue or bodily fluid, see p. 77 lines 7-8. Sheppard and Foley teach using antibodies labeled with radioactive or enzymatic markers, see p. 75, lines 15-19 and p. 68 lines 9-21.

17. If applicant disagrees with any rejection set forth in this office action based on examiner's establishment of a priority date of May 20, 2005 for the instantly claimed application serial number 10/537,002, applicant is invited to submit evidence pointing to the serial number, page and line where support can be found establishing an earlier priority date.

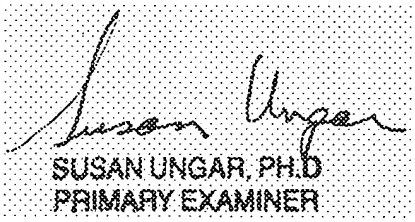
18. No claims are allowed.

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Peter J. Reddig whose telephone number is (571) 272-9031. The examiner can normally be reached on M-F 8:30 a.m.-5:00 p.m..

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Siew can be reached on (571) 272-0787. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Peter J. Reddig, Ph.D.
Examiner
Art Unit 1642



PJR

Appendix I

SCORE Search Results Details for Application 10537002 and Search Result us-10-537-002-7.n2p.rag.

This page gives you Search Results detail for the Application 10537002 and Search Result us-10-537-002-7.n2p.rag.

start | next page

Go Back to previous page

```
                          GenCore version 5.1.9
                    Copyright (c) 1993 - 2006  Biocceleration Ltd.


OM nucleic - protein search, using frame_plus_n2p model

Run on:          September 25, 2006, 23:11:08 ; Search time 20.1 Seconds
                                        (without alignments)
                                        5363.763 Million cell updates/sec

Title:           US-10-537-002-7
Perfect score:   1560
Sequence:        1 atggccgtgactgcctgtca.........ccaagcacgactatgtgtaa 786

Scoring table:   BLOSUM62
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                 Ygapop 10.0 , Ygapext  0.5
                 Fgapop  6.0 , Fgapext  7.0
                 Delop   6.0 , Delext   7.0

Searched:        2589679 seqs, 457216429 residues

Total number of hits satisfying chosen parameters:      5179358

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
                 Maximum Match 100%
                 Listing first 200 summaries

Command line parameters:
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-UNITS=bits -START=1 -END=-1 -MATRIX=blosum62 -TRANS=human40.cdi -LIST=200
-DOCALIGN=200 -THR_SCORE=pct -THR_MAX=100 -THR_MIN=0 -ALIGN=50 -MODE=LOCAL
-OUTFMT=pto -NORM=ext -HEAPSIZE=500 -MINLEN=0 -MAXLEN=2000000000 -HOST=abss05h
-USER=US10537002_@CGN_1_1_165_@runat_25092006_170501_8353 -NCPU=6 -ICPU=3
-NO_MMAP -NEG_SCORES=0 -WAIT -DSPBLOCK=100 -LONGLOG -DEV_TIMEOUT=120
-WARN_TIMEOUT=30 -THREADS=1 -XGAPOP=10 -XGAPEXT=0.5 -FGAPOP=6 -FGAPEXT=7
-YGAPOP=10 -YGAPEXT=0.5 -DELOP=6 -DELEXT=7

Database :       A_Geneseq_8:*
                 1:  geneseqp1980s:*
                 2:  geneseqp1990s:*
                 3:  geneseqp2000s:*
                 4:  geneseqp2001s:*
                 5:  geneseqp2002s:*
                 6:  geneseqp2003as:*
                 7:  geneseqp2003bs:*
                 8:  geneseqp2004s:*
```

Appendix I

```
Db         21 IleIleAlaAlaThrCysMetAspGlnTrpSerThrGlnAspLeuTyrAsnAsnProVal 40

Qy        121 ACAGCTGTTTTCAACTACCAGGGGCTGTGGCGCTCCTGTGTCCGAGAGAGCTCTGGCTTC 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         41 ThrAlaValPheAsnTyrGlnGlyLeuTrpArgSerCysValArgGluSerSerGlyPhe 60

Qy        181 ACCGAGTGCCGGGGCTACTTCACCCTGCTGGGGCTGCCAGCCATGCTGCAGGCAGTGCGA 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 ThrGluCysArgGlyTyrPheThrLeuLeuGlyLeuProAlaMetLeuGlnAlaValArg 80

Qy        241 GCCCTGATGATCGTAGGCATCGTCCTGGGTGCCATTGGCCTCCTGGTATCCATCTTTGCC 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         81 AlaLeuMetIleValGlyIleValLeuGlyAlaIleGlyLeuLeuValSerIlePheAla 100

Qy        301 CTGAAATGCATCCGCATTGGCAGCATGGAGGACTCTGCCAAAGCCAACATGACACTGACC 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        101 LeuLysCysIleArgIleGlySerMetGluAspSerAlaLysAlaAsnMetThrLeuThr 120

Qy        361 TCCGGGATCATGTTCATTGTCTCAGGTCTTTGTGCAATTGCTGGAGTGTCTGTGTTTGCC 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SerGlyIleMetPheIleValSerGlyLeuCysAlaIleAlaGlyValSerValPheAla 140

Qy        421 AACATGCTGGTGACTAACTTCTGGATGTCCACAGCTAACATGTACACCGGCATGGGTGGG 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        141 AsnMetLeuValThrAsnPheTrpMetSerThrAlaAsnMetTyrThrGlyMetGlyGly 160

Qy        481 ATGGTGCAGACTGTTCAGACCAGGTACACATTTGGTGCGGCTCTGTTCGTGGGCTGGGTC 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        161 MetValGlnThrValGlnThrArgTyrThrPheGlyAlaAlaLeuPheValGlyTrpVal 180

Qy        541 GCTGGAGGCCTCACACTAATTGGGGGTGTGATGATGTGCATCGCCTGCCGGGGCCTGGCA 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 AlaGlyGlyLeuThrLeuIleGlyGlyValMetMetCysIleAlaCysArgGlyLeuAla 200

Qy        601 CCAGAAGAAACCAACTACAAAGCCGTTTCTTATCATGCCTCAGGCCACAGTGTTGCCTAC 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        201 ProGluGluThrAsnTyrLysAlaValSerTyrHisAlaSerGlyHisSerValAlaTyr 220

Qy        661 AAGCCTGGAGGCTTCAAGGCCAGCACTGGCTTTGGGTCCAACACCAAAAACAAGAAGATA 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        221 LysProGlyGlyPheLysAlaSerThrGlyPheGlySerAsnThrLysAsnLysLysIle 240

Qy        721 TACGATGGAGGTGCCCGCACAGAGGACGAGGTACAATCTTATCCTTCCAAGCACGACTAT 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 TyrAspGlyGlyAlaArgThrGluAspGluValGlnSerTyrProSerLysHisAspTyr 260

Qy        781 GTG 783
              |||
Db        261 Val 261
```

RESULT 3
AAE07051

```
ID   AAE07051 standard; protein; 261 AA.
XX
AC   AAE07051;
XX
DT   16-OCT-2001  (first entry)
XX
DE   Human gene 1 encoded secreted protein HTPFX16, SEQ ID NO:68.
XX
KW   Human secreted protein; proliferative disorder; cancer; tumour;
KW   foetal abnormality; developmental abnormality; haematopoietic disorder;
KW   immune system disorder; AIDS; autoimmune disease; rheumatoid arthritis;
KW   inflammation; allergy; neurological disorder; Alzheimer's disease;
KW   Parkinson's disease; cognitive disorder; schizophrenia; asthma;
KW   skin disorder; psoriasis; sepsis; diabetes; atherosclerosis;
KW   cardiovascular disorder; angiogenic disorder; kidney disorder;
```

Appendix 1

KW gastrointestinal disorder; pregnancy-related disorder;
KW endocrine disorder; infection; wound healing; vulnerary; cell culture;
KW chemotaxis; food additive; gene therapy; binding partner identification.
XX
OS Homo sapiens.
XX
FH Key Location/Qualifiers
FT Peptide 1. .23
FT /label= Signal_peptide
FT Protein 24. .261
FT /label= Mature_human_secreted_protein
XX
PN WO200154708-A1.
XX
PD 02-AUG-2001.
XX
PF 17-JAN-2001; 2001WO-US001434.
XX
PR 31-JAN-2000; 2000US-0179065P.
PR 04-FEB-2000; 2000US-0180628P.
PR 18-AUG-2000; 2000US-0226279P.
PR 05-DEC-2000; 2000US-0251988P.
PR 05-JAN-2001; 2001US-0259678P.
XX
PA (HUMA-) HUMAN GENOME SCI INC.
XX
PI Rosen CA, Komatsoulis GA, Baker KP, Birse CE, Soppet DR;
PI Olsen HS, Moore PA, Wei P, Ebner R, Duan DR, Shi Y, Choi GH;
PI Fiscella M, Ni J, Ruben SM, Barash SC;
XX
DR WPI; 2001-488743/53.
DR N-PSDB; AAD13345.
XX
PT New isolated nucleic acids and polypeptides, useful for diagnosing,
PT treating and/or preventing human diseases and disorders.
XX
PS Claim 11; Page 487-488; 558pp; English.
XX
CC AAD13345-AAD13401 represent cDNAs corresponding to 22 human secreted
CC protein genes, and AAE07051-AAE07105 represent the proteins they encode.
CC AAE07106-AAE07129 represent human secreted protein fragments or variants.
CC The genes and their secreted proteins are useful for preventing, treating
CC or ameliorating medical conditions, e.g., by protein or gene therapy.
CC Pathological conditions can be diagnosed by determining the amount of the
CC new protein in a sample or by determining the presence of mutations in
CC the new genes. Specific uses are described for each of the 22 genes,
CC based on the tissues in which they are most highly expressed, and include
CC developing products for the diagnosis or treatment of proliferative
CC disorders, cancer, tumours, foetal and developmental abnormalities,
CC haematopoietic disorders, diseases of the immune system, AIDS, autoimmune
CC diseases (e.g., rheumatoid arthritis), inflammation, allergies,
CC neurological disorders (e.g., Alzheimer's disease, Parkinson's disease),
CC cognitive disorders, schizophrenia, asthma, skin disorders (e.g.,
CC psoriasis), sepsis, diabetes, atherosclerosis, cardiovascular disorders,
CC angiogenic disorders, kidney disorders, gastrointestinal disorders,
CC pregnancy-related disorders, endocrine disorders, and infections. The
CC proteins can also be used to aid wound healing and epithelial cell
CC proliferation, to prevent skin aging due to sunburn, to maintain organs
CC before transplantation, for supporting cell culture of primary tissues,
CC to regenerate tissues, to identify their cognate ligands or binding
CC partners, and in chemotaxis, and can be used as a food additive or
CC preservative to modify storage properties. Antibodies specific for a
CC protein of the invention can be used in alleviating symptoms associated
CC with the disorders mentioned above, and in diagnostic immunoassays e.g.,
CC radioimmunoassay or enzyme linked immunosorbent assay (ELISA). The
CC present sequence represents a human secreted protein of the invention
XX

Appendix I

SQ Sequence 261 AA;

Alignment Scores:

Pred. No.:	5.81e-116	Length:	261
Score:	1350.00	Matches:	260
Percent Similarity:	99.6%	Conservative:	0
Best Local Similarity:	99.6%	Mismatches:	1
Query Match:	86.5%	Indels:	0
DB:	4	Gaps:	0

US-10-537-002-7 (1-786) x AAE07051 (1-261)

```
Qy          1 ATGGCCGTGACTGCCTGTCAGGGCTTGGGGTTCGTGGTTTCACTGATTGGGATTGCGGGC  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MetAlaValThrAlaCysGlnGlyLeuGlyPheValValSerLeuIleGlyIleAlaGly  20

Qy         61 ATCATTGCTGCCACCTGCATGGACCAGTGGAGCACCCAAGACTTGTACAACAACCCCGTA 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         21 IleIleAlaAlaThrCysMetAspGlnTrpSerThrGlnAspLeuTyrAsnAsnProVal  40

Qy        121 ACAGCTGTTTTCAACTACCAGGGGCTGTGGCGCTCCTGTGTCCGAGAGAGCTCTGGCTTC 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         41 ThrAlaValPheAsnTyrGlnGlyLeuTrpArgSerCysValArgGluSerSerGlyPhe  60

Qy        181 ACCGAGTGCCGGGGCTACTTCACCCTGCTGGGGCTGCCAGCCATGCTGCAGGCAGTGCGA 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 ThrGluCysArgGlyTyrPheThrLeuLeuGlyLeuProAlaMetLeuGlnAlaValArg  80

Qy        241 GCCCTGATGATCGTAGGCATCGTCCTGGGTGCCATTGGCCTCCTGGTATCCATCTTTGCC 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         81 AlaLeuMetIleValGlyIleValLeuGlyAlaIleGlyLeuLeuValSerIlePheAla 100

Qy        301 CTGAAATGCATCCGCATTGGCAGCATGGAGGACTCTGCCAAAGCCAACATGACACTGACC 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        101 LeuLysCysIleArgIleGlySerMetGluAspSerAlaLysAlaAsnMetThrLeuThr 120

Qy        361 TCCGGGATCATGTTCATTGTCTCAGGTCTTTGTGCAATTGCTGGAGTGTCTGTGTTTGCC 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SerGlyIleMetPheIleValSerGlyLeuCysAlaIleAlaGlyValSerValPheAla 140

Qy        421 AACATGCTGGTGACTAACTTCTGGATGTCCACAGCTAACATGTACACCGGCATGGGTGGG 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        141 AsnMetLeuValThrAsnPheTrpMetSerThrAlaAsnMetTyrThrGlyMetGlyGly 160

Qy        481 ATGGTGCAGACTGTTCAGACCAGGTACACATTTGGTGCGGCTCTGTTCGTGGGCTGGGTC 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        161 MetValGlnThrValGlnThrArgTyrThrPheGlyAlaAlaLeuPheValGlyTrpVal 180

Qy        541 GCTGGAGGCCTCACACTAATTGGGGGTGTGATGATGTGCATCGCCTGCCGGGGCCTGGCA 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 AlaGlyGlyLeuThrLeuIleGlyGlyValMetMetCysIleAlaCysArgGlyLeuAla 200

Qy        601 CCAGAAGAAACCAACTACAAAGCCGTTTCTTATCATGCCTCAGGCCACAGTGTTGCCTAC 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        201 ProGluGluThrAsnTyrLysAlaValSerTyrHisAlaSerGlyHisSerValAlaTyr 220

Qy        661 AAGCCTGGAGGCTTCAAGGCCAGCACTGGCTTTGGGTCCAACACCAAAAACAAGAAGATA 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        221 LysProGlyGlyPheLysAlaSerThrGlyPheGlySerAsnThrLysAsnLysLysArg 240

Qy        721 TACGATGGAGGTGCCCGCACAGAGGACGAGGTACAATCTTATCCTTCCAAGCACGACTAT 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 TyrAspGlyGlyAlaArgThrGluAspGluValGlnSerTyrProSerLysHisAspTyr 260

Qy        781 GTG 783
              |||
Db        261 Val 261
```

Appendix 2

SCORE Search Results Details for Application 10537002 and Search Result us-10-537-002-117_copy_10_816.n2p.50aligns.rag.

This page gives you Search Results detail for the Application 10537002 and Search Result us-10-537-002-117_copy_10_816.n2p.50aligns.rag.

start | next page

Go Back to previous page

```
                        GenCore version 5.1.9
                  Copyright (c) 1993 - 2006  Biocceleration Ltd.


OM nucleic - protein search, using frame_plus_n2p model

Run on:         September 26, 2006, 17:18:19 ; Search time 34.5 Seconds
                                        (without alignments)
                                        3208.467 Million cell updates/sec

Title:          US-10-537-002-117_COPY_10_816
Perfect score:  1604
Sequence:       1 atgtccaccaccacatgcca.....gctctaagacctctcagcac 807

Scoring table:  BLOSUM62
                Xgapop 10.0 , Xgapext  0.5
                Ygapop 10.0 , Ygapext  0.5
                Fgapop  6.0 , Fgapext  7.0
                Delop   6.0 , Delext   7.0

Searched:       2589679 seqs, 457216429 residues

Total number of hits satisfying chosen parameters:      5179358

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
                 Maximum Match 100%
                 Listing first 150 summaries

Command line parameters:
-MODEL=frame+_n2p.model -DEV=xlp
-Q=/abss/ABSSWEB_spool/US10537002/runat_26092006_134901_19850/app_query.fasta_1
-DB=A_Geneseq -QFMT=fastan -SUFFIX=n2p.50aligns.rag -MINMATCH=0.1 -LOOPCL=0
-LOOPEXT=0 -UNITS=bits -START=1 -END=-1 -MATRIX=blosum62 -TRANS=human40.cdi
-LIST=150 -DOCALIGN=200 -THR_SCORE=pct -THR_MAX=100 -THR_MIN=0 -ALIGN=50
-MODE=LOCAL -OUTFMT=pto -NORM=ext -HEAPSIZE=500 -MINLEN=0 -MAXLEN=2000000000
-HOST=abss02p -USER=US10537002_@CGN_1_1_364_@runat_26092006_134901_19850
-NCPU=6 -ICPU=3 -NO_MMAP -NEG_SCORES=0 -WAIT -DSPBLOCK=100 -LONGLOG
-DEV_TIMEOUT=120 -WARN_TIMEOUT=30 -THREADS=1 -XGAPOP=10 -XGAPEXT=0.5 -FGAPOP=6
-FGAPEXT=7 -YGAPOP=10 -YGAPEXT=0.5 -DELOP=6 -DELEXT=7

Database :      A_Geneseq_8:*
                1:  geneseqp1980s:*
                2:  geneseqp1990s:*
                3:  geneseqp2000s:*
                4:  geneseqp2001s:*
                5:  geneseqp2002s:*
                6:  geneseqp2003as:*
                7:  geneseqp2003bs:*
                8:  geneseqp2004s:*
```

Appendix 2

```
Db         81 AlaLeuMetIleValGlyIleValLeuGlyAlaIleGlyLeuLeuValSerIlePheAla 100

Qy        301 CTGAAATGCATCCGCATTGGCAGCATGGAGGACTCTGCCAAAGCCAACATGACACTGACC 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        101 LeuLysCysIleArgIleGlySerMetGluAspSerAlaLysAlaAsnMetThrLeuThr 120

Qy        361 TCCGGGATCATGTTCATTGTCTCAGGTCTTTGTGCAATTGCTGGAGTGTCTGTGTTTGCC 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SerGlyIleMetPheIleValSerGlyLeuCysAlaIleAlaGlyValSerValPheAla 140

Qy        421 AACATGCTGGTGACTAACTTCTGGATGTCCACAGCTAACATGTACACCGGCATGGGTGGG 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        141 AsnMetLeuValThrAsnPheTrpMetSerThrAlaAsnMetTyrThrGlyMetGlyGly 160

Qy        481 ATGGTGCAGACTGTTCAGACCAGGTACACATTTGGTGCGGCTCTGTTCGTGGGCTGGGTC 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        161 MetValGlnThrValGlnThrArgTyrThrPheGlyAlaAlaLeuPheValGlyTrpVal 180

Qy        541 GCTGGAGGCCTCACACTAATTGGGGGTGTGATGATGTGCATCGCCTGCCGGGGCCTGGCA 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 AlaGlyGlyLeuThrLeuIleGlyGlyValMetMetCysIleAlaCysArgGlyLeuAla 200

Qy        601 CCAGAAGAAACCAACTACAAAGCCGTTTCTTATCATGCCTCAGGCCACAGTGTTGCCTAC 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        201 ProGluGluThrAsnTyrLysAlaValSerTyrHisAlaSerGlyHisSerValAlaTyr 220

Qy        661 AAGCCTGGAGGCTTCAAGGCCAGCACTGGCTTTGGGTCCAACACCAAAAACAAGAAGATA 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        221 LysProGlyGlyPheLysAlaSerThrGlyPheGlySerAsnThrLysAsnLysLysIle 240

Qy        721 TACGATGGAGGTGCCCGCACAGAGGACGAGGTACAATCTTATCCTTCCAAGCACGACTAT 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 TyrAspGlyGlyAlaArgThrGluAspGluValGlnSerTyrProSerLysHisAspTyr 260

Qy        781 GTG 783
              |||
Db        261 Val 261
```

```
RESULT 2
AAY70675
ID   AAY70675 standard; protein; 261 AA.
XX
AC   AAY70675;
XX
DT   18-JUL-2000  (first entry)
XX
DE   Human stomach protein zsig28.
XX
KW   Human; stomach; zsig28 protein; chromosome 3q22.1-3q22.2; gene therapy;
KW   claudin; oligodendrocyte-specific protein; OSP; apoptosis; RVP.1;
KW   rat androgen-withdrawal apoptosis protein; growth factor receptor;
KW   cell-cell signalling molecule; cytostatic; antibacterial; food poisoning;
KW   Botulism; diarrhoea; inflammation; cramping; cancer; gastric ulcer;
KW   diagnosis; prevention; treatment.
XX
OS   Homo sapiens.
XX
FH   Key             Location/Qualifiers
FT   Peptide         1. .23
FT                   /label= Secretory_signal_peptide
FT   Protein         24. .261
FT                   /label= Mature_zsig28_protein
FT   Region          24. .82
FT                   /label= Region_1
FT                   /note= "useful as antigenic epitope for antibody
FT                   production"
FT   Region          48. .54
```

Appendix 2

```
FT                   /label= Motif_1
FT                   /note= "Conserved and low variance motif"
FT   Region          77. .82
FT                   /label= Motif_2
FT                   /note= "Conserved and low variance motif"
FT   Domain          83. .100
FT                   /label= Transmembrane_domain
FT   Region          101. .122
FT                   /label= Region_2
FT                   /note= "useful as antigenic epitope for antibody
FT                   production"
FT   Domain          123. .140
FT                   /label= Transmembrane_domain
FT   Region          141. .174
FT                   /label= Region_3
FT                   /note= "useful as antigenic epitope for antibody
FT                   production"
FT   Region          174. .180
FT                   /label= Motif_3
FT                   /note= "Conserved and low variance motif"
FT   Domain          175. .192
FT                   /label= Transmembrane_domain
FT   Region          184. .189
FT                   /label= Motif_4
FT                   /note= "Conserved and low variance motif"
FT   Region          193. .261
FT                   /label= Region_4
FT                   /note= "hydrophilic region useful as antigenic epitope
FT                   for antibody production"
XX
PN   WO200015659-A2.
XX
PD   23-MAR-2000.
XX
PF   14-SEP-1999;   99WO-US021023.
XX
PR   16-SEP-1998;   98US-00154444.
XX
PA   (ZYMO ) ZYMOGENETICS INC.
XX
PI   Sheppard PO,  Foley KP;
XX
DR   WPI; 2000-271379/23.
DR   N-PSDB; AAZ52249.
XX
PT   New isolated polynucleotide encoding a stomach zsig28 polypeptide used
PT   for diagnosis, prevention and treatment of stomach disorders caused by
PT   bacteria, gastric ulcers or cancer.
XX
PS   Claim 12; Page 113-114; 121pp; English.
XX
CC   The present sequence is a stomach protein zsig28 from human lung library.
CC   The zsig28 gene is located at 3q22.1-3q22.2 region of human chromosome 3.
CC   The protein shows homology to a diverse family of receptor proteins
CC   containing e.g. human claudin 1 and 2, human and murine oligodendrocyte-
CC   specific protein (OSP) and rat androgen-withdrawal apoptosis protein
CC   RVP.1. It is thought to be a cell-cell signalling molecule, a growth
CC   factor receptor or extracellular matrix associated protein with growth
CC   factor hormone activity and may be involved in an apoptotic cellular
CC   pathway. The protein may act as an anti-microbial agent and may bind
CC   toxins produced by bacteria which cause food poisoning, Botulism, severe
CC   diarrhoea, inflammation and cramping. zsig28 agonists are useful for
CC   promoting apoptosis in cells over-expressing zsig28 e.g. in cancer cells.
CC   They are also useful for stimulating cell growth or differentiation.
CC   Altered levels of zsig28 protein in a test sample such as saliva, serum,
CC   sweat or biopsy can be monitored as an indication of digestive function,
CC   gastric ulcer or cancer. zsig28 expression can be used as a
```

Appendix 2

CC differentiation marker to determine the stage of tumour or cell maturity,
CC particularly in epithelial cells. Polynucleotides encoding zsig28 can be
CC used in gene therapy applications to increase or inhibit zsig28 activity
XX
SQ Sequence 261 AA;

Alignment Scores:

Pred. No.:	2.63e-113	Length:	261
Score:	1357.00	Matches:	261
Percent Similarity:	100.0%	Conservative:	0
Best Local Similarity:	100.0%	Mismatches:	0
Query Match:	84.6%	Indels:	0
DB:	3	Gaps:	0

US-10-537-002-117_COPY_10_816 (1-807) x AAY70675 (1-261)

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Qy          1 ATGTCCACCACCACATGCCAAGTGGTGGCGTTCCTCCTGTCCATCCTGGGGCTGGCCGGC  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MetSerThrThrThrCysGlnValValAlaPheLeuLeuSerIleLeuGlyLeuAlaGly  20

Qy         61 TGCATCGCGGCCACCGGGATGGACATGTGGAGCACCCAGGACCTGTACGACAACCCCGTC 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         21 CysIleAlaAlaThrGlyMetAspMetTrpSerThrGlnAspLeuTyrAspAsnProVal  40

Qy        121 ACCTCCGTGTTCCAGTACGAAGGGCTCTGGAGGAGCTGCGTGAGGCAGAGTTCAGGCTTC 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         41 ThrSerValPheGlnTyrGluGlyLeuTrpArgSerCysValArgGlnSerSerGlyPhe  60

Qy        181 ACCGAATGCAGGCCCTATTTCACCATCCTGGGACTTCCAGCCATGCTGCAGGCAGTGCGA 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 ThrGluCysArgProTyrPheThrIleLeuGlyLeuProAlaMetLeuGlnAlaValArg  80

Qy        241 GCCCTGATGATCGTAGGCATCGTCCTGGGTGCCATTGGCCTCCTGGTATCCATCTTTGCC 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         81 AlaLeuMetIleValGlyIleValLeuGlyAlaIleGlyLeuLeuValSerIlePheAla 100

Qy        301 CTGAAATGCATCCGCATTGGCAGCATGGAGGACTCTGCCAAAGCCAACATGACACTGACC 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        101 LeuLysCysIleArgIleGlySerMetGluAspSerAlaLysAlaAsnMetThrLeuThr 120

Qy        361 TCCGGGATCATGTTCATTGTCTCAGGTCTTTGTGCAATTGCTGGAGTGTCTGTGTTTGCC 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SerGlyIleMetPheIleValSerGlyLeuCysAlaIleAlaGlyValSerValPheAla 140

Qy        421 AACATGCTGGTGACTAACTTCTGGATGTCCACAGCTAACATGTACACCGGCATGGGTGGG 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        141 AsnMetLeuValThrAsnPheTrpMetSerThrAlaAsnMetTyrThrGlyMetGlyGly 160

Qy        481 ATGGTGCAGACTGTTCAGACCAGGTACACATTTGGTGCGGCTCTGTTCGTGGGCTGGGTC 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        161 MetValGlnThrValGlnThrArgTyrThrPheGlyAlaAlaLeuPheValGlyTrpVal 180

Qy        541 GCTGGAGGCCTCACACTAATTGGGGGTGTGATGATGTGCATCGCCTGCCGGGGCCTGGCA 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 AlaGlyGlyLeuThrLeuIleGlyGlyValMetMetCysIleAlaCysArgGlyLeuAla 200

Qy        601 CCAGAAGAAACCAACTACAAAGCCGTTTCTTATCATGCCTCAGGCCACAGTGTTGCCTAC 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        201 ProGluGluThrAsnTyrLysAlaValSerTyrHisAlaSerGlyHisSerValAlaTyr 220

Qy        661 AAGCCTGGAGGCTTCAAGGCCAGCACTGGCTTTGGGTCCAACACCAAAAACAAGAAGATA 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        221 LysProGlyGlyPheLysAlaSerThrGlyPheGlySerAsnThrLysAsnLysLysIle 240

Qy        721 TACGATGGAGGTGCCCGCACAGAGGACGAGGTACAATCTTATCCTTCCAAGCACGACTAT 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 TyrAspGlyGlyAlaArgThrGluAspGluValGlnSerTyrProSerLysHisAspTyr 260
```

Appendix 2

```
Qy        781 GTG 783
              |||
Db        261 Val 261

RESULT 3
AAY92235
ID   AAY92235 standard; protein; 261 AA.
XX
AC   AAY92235;
XX
DT   10-AUG-2000  (first entry)
XX
DE   Claudin homologue from clone 3224646 cDNA.
XX
KW   Clone 3224646; claudin; homologue; cytostatic; anti-HIV;
KW   immunosuppressive; antiallergic; antiinfective; antiinflammatory;
KW   antiarthritic; antiarteriosclerotic; vasotropic; neuroprotective;
KW   nootropic; dermatological; tranquilizer; vulnerary.
XX
OS   Homo sapiens.
XX
FH   Key             Location/Qualifiers
FT   Peptide         1. .23
FT                   /label= signal_peptide
FT   Protein         23. .261
FT                   /label= mature_protein
XX
PN   WO200020447-A2.
XX
PD   13-APR-2000.
XX
PF   06-OCT-1999;   99WO-US023294.
XX
PR   06-OCT-1998;   98US-0103195P.
PR   05-OCT-1999;   99US-00412231.
XX
PA   (CURA-) CURAGEN CORP.
XX
PI   Shimkets RA;
XX
DR   WPI; 2000-303741/26.
DR   N-PSDB; AAA09116, AAA09121.
XX
PT   Nucleic acids encoding polypeptides with syncline-like, claudin-like or
PT   cytokine-like activity, useful for treating diseases including cancer,
PT   Alzheimer's and atherosclerosis.
XX
PS   Claim 20; Fig 3A; 118pp; English.
XX
CC   Clone 3223867 encodes a polypeptide that has homology to claudin-1, which
CC   is an integral membrane protein found in tight junctions. The sequences
CC   are useful for treatment of diseases such as cancer, immune disorders,
CC   autoimmune disease, acquired immune deficiency syndrome (AIDS),
CC   transplant rejection, allergy, infection by a pathological agent or
CC   organism, inflammatory disorders, arthritis, a haematopoietic disorder, a
CC   skin disorder, atherosclerosis, restenosis, a neurological disease,
CC   Alzheimer's disease, trauma, spinal cord injury and skeletal disorders
XX
SQ   Sequence 261 AA;

Alignment Scores:
Pred. No.:                2.63e-113      Length:          261
Score:                    1357.00        Matches:         261
Percent Similarity:       100.0%         Conservative:    0
Best Local Similarity:    100.0%         Mismatches:      0
Query Match:              84.6%          Indels:          0
```